GAMETIC AND OBSERVED RATIOS IN DROSOPHILA

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THE populations and families with which the geneticist deals are not the real objects of his investigation; for him, the distribution of characters is only an index of the preceding distribution of genes in gametes. But the whole course of embryonic development, with heavy mortality possible at every step, has intervened between the individuals that he classifies and the gametes from which they came. The observed classes correspond accurately to the original gametic series only in case this mortality is indiscriminate—that is, only if there is no differential viability.

In the breeding work with *Drosophila* there has been a continual effort to eliminate distortion in the ratios, which depends largely upon: (1) the extent of the mortality involved, this being characteristic in amount for each mutant type and character combination, (2) the suitability of the culture media and conditions, and (3) the competition when the number of developing individuals is in excess of the optimum number for the available food supply.

The problem of over-crowding (3) is simplest of solution, though over-crowding was the largest source of disturbance in most of the early work, as well as in some of the later. The remedy is, in the first place, to limit the number of eggs per culture to the output of a single female. No mass-cultures should be raised in experiments in which the ratios among the offspring are of importance. In the second place, as the larvæ grow larger and also increase in number with each day's output of eggs, the competition becomes intensified throughout the later stages of the culture. To meet this increasing demand, there must be fresh supplies of food, or enough food must be provided at the start so that even at the end there is sufficient for free development of all larvæ. In point of economy it is better to concentrate on a few cultures that are liberally supplied than to raise a greater

number that would mean optimum conditions for none and doubt concerning the reliability of all.

The main problem in connection with the environment (2) is to find a kind of food that will allow full development of even very weak classes. It is in this field that the greatest changes in method have been made. For some years—from 1910 to 1916—some modification of the fermented-banana method of preparing food was followed. Ripe sound bananas were peeled, and the pulp left for about 24 hours in a liquid containing yeast. This liquid was usually the fermented juice from the previous lot of bananas. About 25 grams of this fermented banana was put in the bottom of a culture bottle and covered with absorbent paper.

It was suspected that the real food of the larvæ was not primarily the banana but was rather the yeast cells and perhaps also the bacteria, the banana being mainly the culture medium for the yeast. This has been established by the work of Northrop,[1] of Loeb and Northrop,[2] and of Baumberger.[3] In July, 1916, in consultation with Northrop, I started experiments with a view to using as a culture medium standardized solutions, instead of banana. The solution was absorbed and held in a cake in the bottom of the culture bottle by shredded paper toweling, which offered extensive surface for the growth of the yeast. This method was unsuccessful; the flies laid few eggs and these were often overgrown by the yeast and killed. Esters and other chemicals with fruit odors did not lead to greater egg production. Perhaps better success with culture solutions would be obtained in supplementing and modifying banana methods.

The banana method was modified with a view to discouraging the growth of moulds and putrefactive bacteria by mild antiseptics or correctives, such as benzoate, thymol, formaldehyde, alcohol, powdered marble for neutralization of excessive acidity, etc. Good results were obtained with alcohol, where several extensive sets of comparative tests seemed to show that about 1.5 per cent. of alcohol in the food was desirable. The most successful alcohol method was roughly as follows: The pulp of sound ripe bananas was weighed and put with an equal number of c.c. of 3 per cent. alcohol in a shallow, covered dish. No yeast was added, since enough wild yeast was usually present. The food

[1] *Jour. Biol. Chem.*, 1917, pp. 181–187.
[2] *Jour. Biol. Chem.*, 1916, pp. 309–312.
[3] *Jour. Exp. Zool.*, 1919, pp. 1–28.

was at its best when it had fermented for about 24 hours. The optimum amount of drained banana was found to be about 25 grams per bottle. This was put upon the bottom of the culture bottle and one gram of paper-toweling strips (about 5. ×.7 cm.) was matted down on the top. Pint culture bottles gave a greater output per pair than halfpints. The alcohol method was used more successfully than the old method during the fall and winter of 1916.

In the spring of 1917 considerable work was done in testing out various media containing starch, sugar, peptone and salts. This method gave good results except that trouble with moulds was greatly increased.

In the autumn of 1916, Dr. R. W. Glaser told me of certain culture-media experiments that Mr. Baumberger and he were carrying out with banana infusions and agar.[4] Dr. Glaser later sent me directions for preparing these media and also some prepared tubes. My tests of the method showed that the amount of food was inadequate for general use, although sufficient for the small number of flies that they wished. I increased the concentration of the media by the addition of sugar, banana flour, etc., but principally by grinding up and using all the pulp of the bananas, instead of using simply the strained juice. A comparison of fresh banana with banana that had been fermented before incorporation showed that the fresh banana was superior. Likewise fresh banana was superior to banana raised to the boiling point at any stage of preparation. It was found that yeast should not be distributed throughout the media. Experiments showed that it is advisable to have a very light seeding of yeast confined to the surface of the solidified media. Also it is well to keep the yeast from the margin as much as possible, since fermentation at the sides and beneath the cake makes the cake break loose and rise. The amount of agar was found to be adequate at 1 per cent.

It was some months before this method was improved so far that it gave better results than those given by the old method or the alcohol method. In the spring of 1917 it was worked out well enough so that it was substituted for the old method in my regular work. By the winter of 1917 it had become quite

[4] *Science*, 1917, pp. 21–22.

generally adopted in the laboratory. Several points have been improved since, so that the procedure at present is as follows:

1. Use bananas that are thoroughly ripe or over-ripe.
2. Peel the bananas and weigh the pulp (100 grams of pulp provides for about four culture bottles).
3. Weigh agar-agar, 2 per cent. of amount of banana.
4. Measure as many c.c. of water as there are grams of banana.
5. Add agar-agar to water and heat until the agar has dissolved. (Complete solution is hastened by the addition of a small amount of fresh water soon after the boiling point has been reached.)
6. While the agar is heating, press the banana through a potato masher or a coarse sieve, and place in readiness the bottles (which should have been previously washed and also preferably steam sterilized). Get ready yeast (Magic Yeast ground up) and paper (absorbent paper, paper toweling cut into 4-fold squares $3'' \times 2''$) and cotton (stoppers may be reused, but should be dry sterilized by enclosing over formalin. Cotton stoppers are better if made rather tight and covered with very soft cheese-cloth).
7. Stir banana into hot agar solution. Mix thoroughly. Mixture should not be heated any longer.
8. With ladle and funnel pour about 50 c.c. of the media into each half-pint or pint milk bottle. (The media should be at least $\frac{3}{4}$ in. thick to stick well.)
9. Sprinkle top lightly with dry yeast.
10. Put in contact with media a 4-fold square of absorbent paper.
11. Stopper with cotton.
12. Use same day. Best to use as soon as cool. Not good after two days.

Flies can be mated in vials and then transferred to the culture bottles at the end of the day. A little food may be kept going by the alcohol method for use in vials, for covering over mould patches in culture bottles, and for refeeding stock cultures.

The distortions in the ratios that arise from mortality characteristic of given mutants and combinations (1) can not be eliminated by direct methods. Fortunately, a large proportion

of the mutants are little if at all below normal in degree of viability; that is, when such mutants are compared with the wild type under identical conditions, the observed ratios show little or no deviations from expectation beyond those due to random sampling. As an example may be mentioned white-ocelli, which is known to have maintained itself with practically undiminished frequency through 175 generations of competition, under unfavorable conditions of culture,[5] with the wild-type. In the main, the mutant races that show normal viability are those whose somatic effects are "slight". Thus, white-ocelli affects the color of the tiny group of three ocelli on the top of the head. The character, though involving so small an area, is perfectly sharp and definite, and under proper conditions of illumination and magnification is fairly easy to classify. The same is true of many other "slight" mutations, such, for example, as speck, cross-veinless, and hairy, which are among the most valuable *Drosophila* mutations. On the other hand, mutants that involve more extensive or manifold changes, such as club, notch, rudimentary, and delta, are also among those poorest in viability. Some of these changes in themselves interfere with the success of the individuals possessing them: flies with "spread" wings or "dachs" legs are liable to become caught in the culture media and die. These changes are also sometimes obviously accompanied by serious internal derangements. In the case of streak, for example, it can be seen that the internal muscles of the thorax are largely replaced by bubbles and blood sinuses. The correlation between inviability and the extent of the visible change is high, but is lessened by the cases in which the internal accompanying changes are of slight disadvantage. Thus, the mutant "pads" resembles "club" very much, and appears to be a greater change in the same direction, but is nevertheless far freer from inviability. Conversely, certain mutants that are usually lethal occasionally do produce offspring, which are then not as strikingly different from the wild-type as some other mutants that have good viability. Lethal-10 very occasionally survives, and the individuals are scarcely to be distinguished from dwarfs of a certain mutant race (dwarfoid) that is little inferior to the wild-type in viability.

[5] *Biol. Bull.*, 1920, pp. 231–236.

The connection between observed character-change and inviability is even more indirect than suggested above. In the *Drosophila* work it is not the comparative viability of adults possessing given character differences that is of the most importance. Even though many of the characters are of such a nature that their possessors would be under a serious handicap in competition, in relatively few cases does this fact lead to alterations in the observed ratios, since the classifications are made usually soon after the flies hatch, *i.e.*, every 24 to 48 hours. It is true that certain mutant forms such as "divergent" and "gull" and "bifid" wings, also "dachs" and "reduplicated" legs tend to become entangled in the culture media and drowned immediately after emergence, so that in these cases the observed ratios are somewhat different from the hatching ratios. There are also a few mutants—mostly semi-lethals—in which the adult is unable to live very long even under the most favorable conditions. Among these may be mentioned "lemon," "apterous," and especially "decrepit." The "decrepit" flies die a few hours after hatching in spite of all care in helping them emerge from the pupa case, in keeping them in quarters not too dry or wet, and in suplying them with suitable food. It would seem that the death of such flies as are obviously weak on hatching is to be referred to difficulties encountered in the pupa stage.

Even inviability arising in the pupal stage, like that in the adult stage, is less general and significant than that in the larval stage. Most of the inviability that affects the ratios of adults is to be referred to differences acting in the larval stage, as is evident from comparative studies of the results of pair and mass cultures and of changes in culture methods that affect only the larval period. The difference between mass and pair cultures is essentially a difference in the number of larvæ that are in competition, the food conditions and the character of the larvæ being at first identical in the compared cultures. It is found that the distortion to the ratios among the adults is roughly proportional to the number of larvæ in competition. How extreme such competition may be is evident from the fact that a point is soon reached after which further increase in the number of mothers brings no increase in the number of progeny and may even result in a decrease. So predominant is the larval stage in its influence upon viability that the chief field of

improvement of culture conditions has been that of the character and methods of use of the food for the larvæ. There are specific viability differences among the larvæ of the different mutant types and combinations. Such viability differences must depend upon differences in the characters of the larvæ, and these, because of the intervening metamorphosis, have little direct relation to the characters of the adult, but are products of the action of the same mutant gene. The high correlation observed between extensive change in adult characters and high degree of inviability must, then, mean that such genes generally cause changes which interfere directly with the success of the larvæ.

Three larval characters are known—the tumor responsible for the death of lethal-7 larvæ, the much shortened larvæ of the mutation "chubby," and a marking on the posterior end, viz., "barette." It is supposed that a high proportion of the larval characters that lead to inviability are differences in internal structures, but some of these might be detected. However, no systematic search for larval characters has been made even in the case of the inviable mutants where such differences are probably present.

As we have stated, the distortion in ratios that arises from inviability, especially inviability originating in the larval stage, can be very materially reduced by improvements in culture media and in methods. Many poorly viable mutants can be made quite generally usable, as, for example, dachs. But when a mutant such as dachs is to be used in linkage determinations, the experiments should be so planned as not to include more than one of these characters. The presence of a single poorly viable character in an experiment does not prevent the calculation of correct crossover values. The complementary classes that do not include the inviable character should be in the same proportions as in the gametic series. Even in cases of mutants completely lethal, the linkage relations of the lethal gene can be calculated accurately from the ratios shown by the other characters of the cross. The classes that include the inviable character are often also usable, but with less certainty that the values are correct. Such values are correct when the presence of the mutant decreases by the same percentage the size of every class in which it occurs. The fewer the mutants involved in an experiment, the greater the likelihood that this

result will follow. Unexpected irregularities in ratios may arise where many mutant characters are distributed in different combinations. These peculiarities of inviability are probably comparable to "specific" and "disproportionate" modifications in eye-color, etc.[6]

If more than one poorly viable mutant is present in a linkage experiment, there is distortion in the ratios due to linkage, and such experiments are either entirely worthless, or are only to be regarded as rough indicators of the real relations. As we saw, if only one inviable mutant is present in a cross, one of each of the pairs of complementary classes remains undisturbed; and correct values can be calculated from them. The presence of a second inviable mutant leaves undisturbed only that class in which neither mutant occurs. The calculation of crossover values under this circumstance is somewhat comparable to solving for two unknowns with a single equation. Solutions can be obtained only by assuming some relationship between the two disturbances. Thus, we may assume that the disturbances are independent; that is, that there is no specific interaction of the kind mentioned above, and the class in which both mutants occur is accordingly of the size that would be expected from the amount of the disturbance present in those classes in which each occurs by itself. On this basis, the crossover values are calculated from the square root of the product of the two complementary non-crossovers, and likewise of the crossover classes, instead of from the sums of such complementary classes.[7] The assumption of independence would be approximately correct in perhaps a majority of crosses in which only two or a few loci are involved. If the disturbances are related—if they tend to neutralize or to exaggerate each other—a correction can still be made by raising an equal number of individuals in the complementary cross. In the two complementary back-crosses, a $\times$ b (repulsion) and a b $\times$ wild-type (coupling), the character combinations that are non-crossover classes in the repulsion experiment are crossovers in the coupling experiment, and vice versa. If the presence of a particular class has given a crossover value too high in the one cross, then it will give a value correspond-

[6] *Jour. Exp. Zool.*, 1919, p. 374.

[7] This geometric mean method was proposed by Muller, who gave an excellent discussion of the difficulties involved in differential viability. (AM. NAT., Vol. L, p. 351 ff.)

ingly too low in the complementary cross, and the mean value will be correct.[8]

Since the effects of inviability are likely to be more pronounced, even disproportionately so, as the number of mutant characters present simultaneously is increased, it is advisable to plan any linkage experiment in which several characters are to be involved, in such a way that the characters are distributed as evenly as possible. The type of back-cross that gives the evenest possible distribution, as well as the smallest proportion of individuals in which the higher combinations occur, is that in which half the mutants have entered the cross from one parent and the other half from the other parent, and in which the mutants are *"alternated"* as regards their positions along the chromosome. Thus, for example, let us consider a back-cross in the third chromosome in which the seven mutants to be used are: roughoid at 0.0; hairy at 25.8; scarlet at 35.1; dichæte at 38.5; pink at 44.6; spineless at 54.2; and ebony-2 at 66.9. The two parents should be roughoid scarlet pink ebony-2, and hairly dichæte spineless; and the formula for the F_1 multiple heterozygote would be:

$$\frac{\text{ru} \quad \text{st} \quad \text{p} \quad \text{e}^2}{\text{h} \quad \text{D} \quad \text{ss}}.$$

The production of an individual possessing all seven characters would require an hexuple crossover, which almost certainly would not occur.[9]

A method that overcomes inviability effects to the greatest extent where many mutants are involved, but which unfortunately requires too great labor for general use, was devised by Muller.[10] F_1 females heterozygous for any number of mutants are crossed, not to the multiple recessive as in the ordinary back-cross, but to the wild-type. Except for the dominant mutants, none of the characters involved appears in the resulting individuals, and hence do not exert injurious effects. These indi-

[8] This "balancing of the inviability" has been discussed at greater length by Bridges, *Jour. Exper. Zool.*, 1915, p. 3 ff.; *Jour. Exper. Zool.*, 1920, pp. 281, 288; by Morgan and Bridges, Carnegie Pub. No. 237, p. 19, 43; and by Muller, AM. NAT., 1916, p. 353.

[9] See Bridges, *Jour. Exper. Zool.*, 1920, p. 295, for discussion and other examples of the "alternated back-cross."

[10] AM. NAT., 1916, p. 354 ff.

viduals have then to be tested singly to determine what recessive characters they carry and hence to what crossover category they belong.

Thus, by improvements (1) in the type of experiment planned, (2) in the culture media and methods used, and (3) in the method of calculation, disturbances in the ratios can usually be held within negligible amounts.

To these indirect methods of obtaining accurate values is to be added one still more important—namely, the discovery of new mutants in which viability is practically normal, and which can be substituted for mutants less satisfactory in that regard. Many of the loci are represented by several mutant allelomorphs, which often are different in viability as in other characteristics. Thus, of the eight cut allelomorphs, or appearances, cut-6 is distinctly the most nearly normal in viability. Likewise, of the five or six allelomorphs of the truncate locus, "dumpty" is the most satisfactory.

In most of the more complex linkage problems, especially those involving linkage-variations or coincidence, the behavior of particular regions of a chromosome is being examined, and the particular loci utilized are only indices of the behavior. What is most essential, therefore, is that there be workable mutant loci distributed rather evenly over all regions of the chromosomes. As the number of mutants in a particular region increases, there is a greater range of choice and greater probability that one or more of the mutants of that region will have normal viability. Thus, bifid (at 7.3) was long the only workable mutant at a favorable distance from the left end of the X-chromosomes. More recently ruby (at 7.5) because of its better viability has displaced bifid from general use, and this in spite of the fact that ruby interferes with the classification of several other eye-colors (especially prune and garnet) while bifid is workable with nearly all other mutants.

In regions less well represented in numbers of mutant loci, a mutant with excellent characteristics may be used rather than one whose position is more favorable but whose other characteristics are poorer. Thus, "humpty" is very favorably located in the second chromosome in the middle of the long region from curved (73.5) to plexus (98.5), but its viability is so poor that in most experiments it is better to leave this section unfollowed than to introduce humpty. There are at present few regions

that are not satisfactorily represented. By far the longest of these in the 25.8 unit interval from roughoid to sepia in the left end-region of the third chromosome. Because of uncertainties in classification, it is not ordinarily possible to use more than two eye-color mutants together in an experiment, and such masking of one character by others affecting the same organ leads to a continued search for combinations of characters that can be handled simultaneously. In general, the slight mutant characters mask each other less than do extreme ones, and these are, usually the least inviable. There is a continual improvement of the working material by the substitution of better mutants.